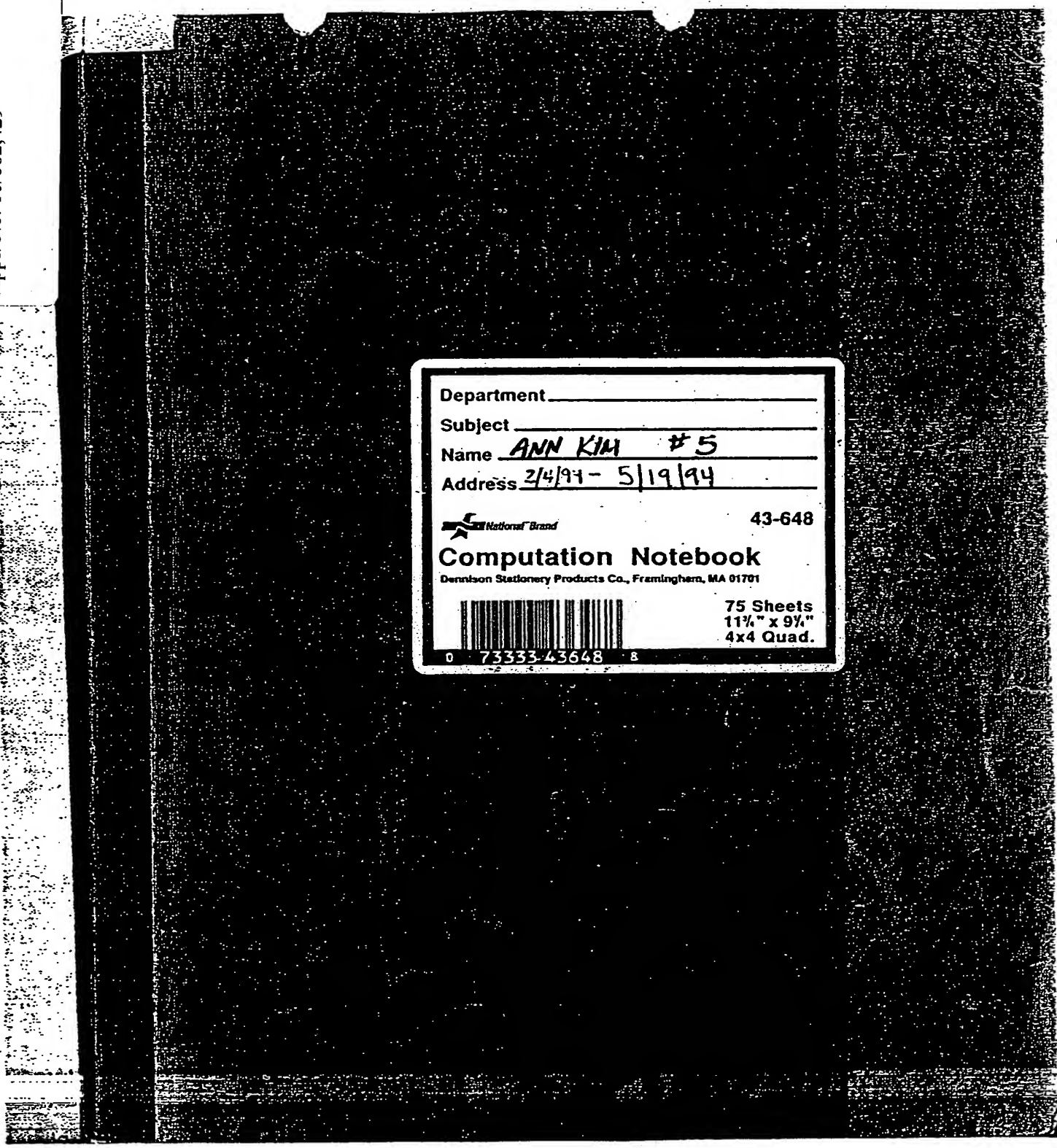
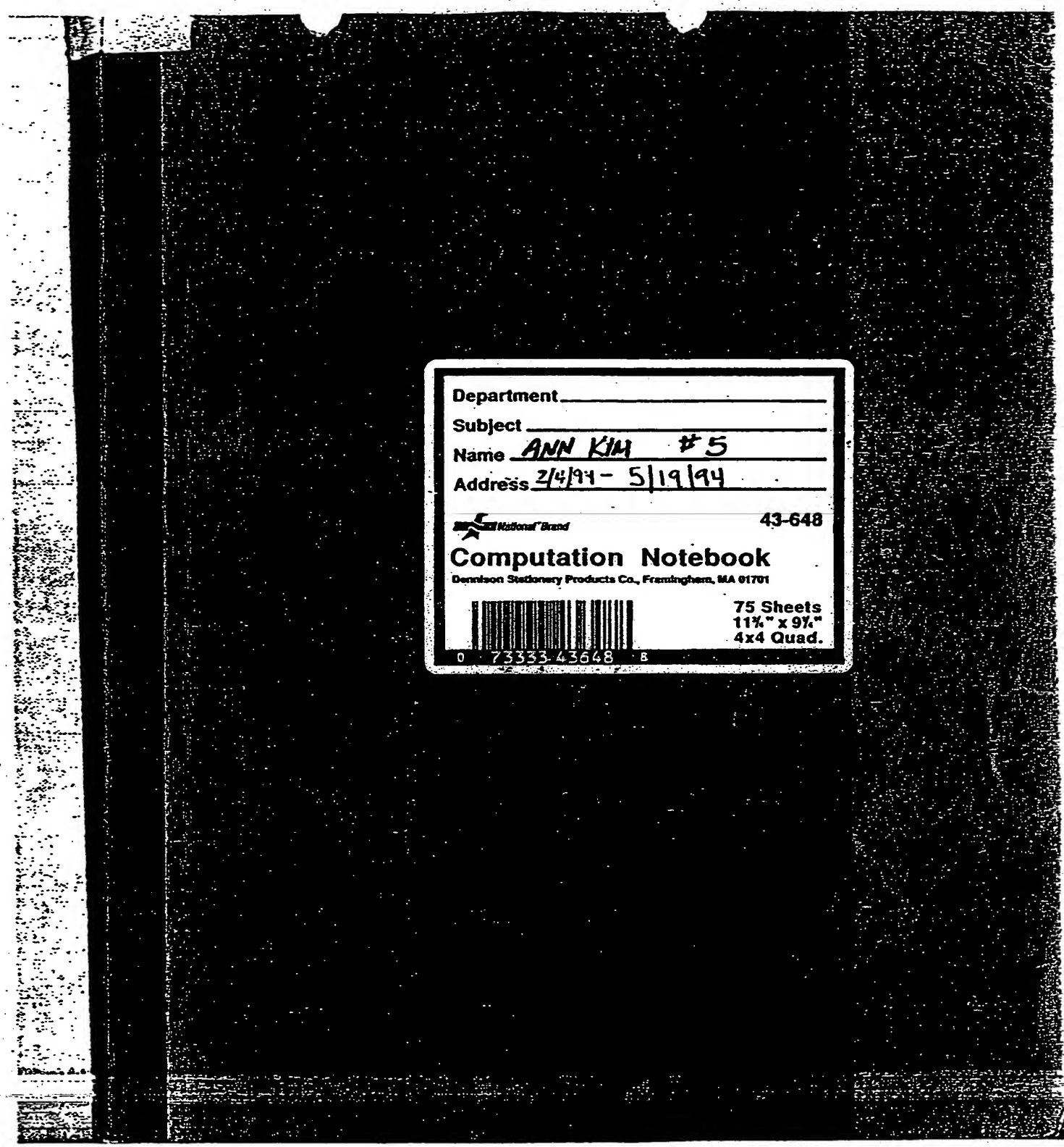
Steven M. Ruben
Appl. No. 10/662,429



BEST AVAILABLE COPY

Ruben EXHIBIT #88



Ruben EXHIBIT 2088
Ruben v. Wiley et al.
Interference No. 105,077
RX 2088

HTPAD05 Screening 1°

Thursday 2/3/94

~~HTPAD05~~ HTPAD05 Screening

~~HTPAD05~~

HTP Lib from Scott Wiessner

1×10^9 pfu/ml

12 plates - 7.2 ml LE392 $OD_{600} = 1.0$
+ 48 ml 1:100 dilution

Incubate 37°C 5 hrs
Cool 4°C 1 hr.
Do lifts

FRIDAY 2/4/94

Denature 0.5N NaOH, 1.5M NaCl

Neutralize 0.5M Tris pH8, 1.5M NaCl

Let air Dry

Monday 2/7/94

Prehybridize in 2x PIPES buffer

42°C incubator

Fragments - HACSK38, HE8AY29, HTPAN08 - R1/Xho 13

FRIDAY 2/4/94

Fragment Prep:

Digest: HACSK38
HE8AY29
HTPAN08

With ECoR1 / XhoI

DNA	10 µl
10x #2	5 µl
10x BSA	5 µl
R1	2
Xho	2
H_2O	26 µl
	50 µl

Incubate 37°C, 2hrs
Run 1-5ul

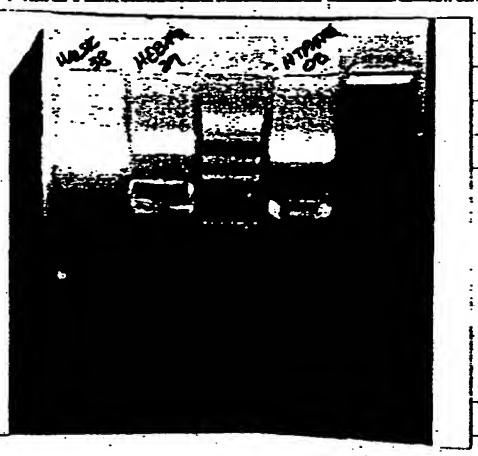
Run on low melt gel + cut out

Transparent gels

Monday 2/7/94

Run on 2% LMPGel
With 1Kb

Run 80V 2hrs – 2½ hrs



Cut out bands:

HALSK38	R1/Xho	~600 bp.
HE8A429	R1/Xho	1.1 Kb
	R1/Xho	1.8 Kb
HTPAW08	R1/Xho	1.1 Kb

Used TRAK kit for Random Prime Rxns.

HTF-NOS Screening

Pg 8

Tuesday 2/8/94

Pour off Stoke

Wash 0.2xSSC ; 0.1% SDS

Wash 3 x 50°C
0.2xSSC ; 0.1% SDS
30min

Put on film
-80°C ON.

Wednesday 2/9/94

Developed film.

Total of 37 ⊕ Clones

1 - 4
2 - 2
3 - 4
4 - 3
5 - 4
6 - 3
7 - 4
8 - 5
9 - 8
10 - 8
11 - 3
12 - 5

Pick plug into
500 µl SM Buffer.
Store 4°C

Do 2° Screening on all ⊕ Clones.

Plate full ? 200µl LE392
plate onto 100mm plate

HTBANO8 Screening

2/15/94 Tuesday

1ml of phage ⇒ 300µl LE392 $OD_{600} = 1.0$.
incubate 37°C 15 min
Plate onto NZY Plate (100mm)
in 5ml LB + 0.75% Agarose
Melted 55°C

Incubate 37°C 15 hours
Place plate at 4°C.

2/16/94 Wednesday

Do plate lifts
Denature: 2 min
Neutralize 5 Min

Prehybridize @ 42°C in 2x PIPES

Random Prime fragment

Low Melting gel fragment	24ml
5x Buffers	10ml
	34ml

Heat 100°C 5 min
Quick Chill; Quick Spin

Add:

5x dATP Buffer	10ml
α^{32}P dATP	5ml
Klenow	1µl
	50µl

Incubate 37°C 15 min

1+1 PANO8 Screening

Pde downloaded 2/16/94

Put through G25 column

Count 1 ul;
2.23 x 10^4 counts/ul

Use 20ul ⇒ 40ml Hyb Buffer;
Boil probe 100°C 5 min in 50ul
Salmon Sperm DNA - Quick Chill

Incubate 42°C ON

Thursday 2/17/94

Wash 0.2xSSC; 0.1%SDS

Wash 3x 55°C
0.2 x SSC, 0.1%SDS
>30 min/wash

Put on film -80°C.

Develop after 3hrs.

FRIDAY 2/18/94

① Clones

~~1268~~

37 ① Clones

~~100 ① Clones~~

Do Mini Preps

	1-3	2-1	3-4	5-2	7-1	8-2	11-1
12-3	1-2	2-2	4-1	5-3	7-2	8-3	11-2
12-4	1-1	3-1	4-2	5-4	7-3	8-4	11-3
12-5	1-4	3-2	4-3	6-2	7-4	6-1	12-1
		3-3	5-1	6-3	6-1	8-5	12-2

HRAND06 2nd Screening

2/22/94 Tuesday

DO Mini Preps

Phage 100 µl
EXA-Host 4 µl
XL-1 Blue OD_{600} = 1.0 - 200 µl

Incubate 37°C 15 min
Add 3 ml LB
Incubate 37°C 2 hrs with aeration

Heat 70°C 20 min
Spin 3K 10 min

Transfer to fresh tubes
Store at 4°C

2/23/94 Wednesday

5 µl → 200 µl SOLR OD_{600} = 1.0

Incubate 37°C 15 min
Plate 100 µl onto LB Amp + IPTG/Xgal

Incubate 37°C O/N

DO PCR on Phage Mini Preps — (see pg 59)

2/24/94 Thursday

Plates over grown
Replate —
Add 10 mM $MgSO_4$ to 1 ml
Incubate 37°C 15 min
Plate 75 µl on LB Amp + IPTG/Xgal
Incubate 37°C ON

(pg 52)

HTLVANO8 (pg 32) 2° Screening

FRIDAY 2/25/94

PCR clones of each into LBt Amp — 96 well Dish.
Incubate 4 hrs 37°C w aeration
Put at 4°C

Monday 2/28/94

Incubate 37°C 2 hrs
Do PCR.

M13F	0.1
M13R	0.1
Culture	1
HTPAN1575	0.1
10x dNTP	2.1
10x PCR Taq	2.1
	0.15
H_2O	15.35
	21

Use HTPANO8 Plasmid DNA as ⊕ Control.

PCR Program 69.

Tuesday 3/1/94

Run 10 µl on gel w. 1 Kb Ladder

H. PANO8 Screening

(pg 32)

Wednesday 2/23/94

PCR Phagemid

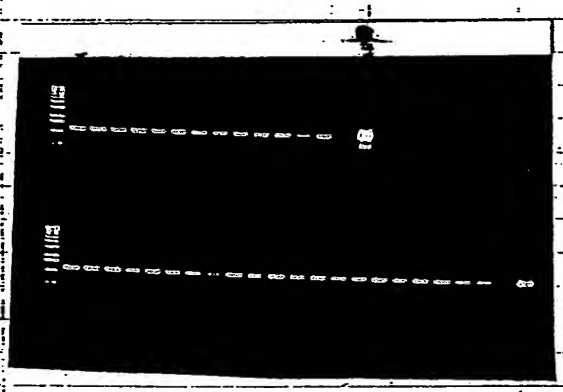
Primer = H7PANO8.6G5

Primer	0.1
M13F	0.1
10x PCR	2.1
10x dN	2.1
Phagmid	1
H_2O	15.45
Taq	0.15
	21.

(+) control H7PANO8 plasmid DNA.

PCR Program #69

Run 10 µl + 1Kb Ladder.



see pg 32

60 HPAN Screening

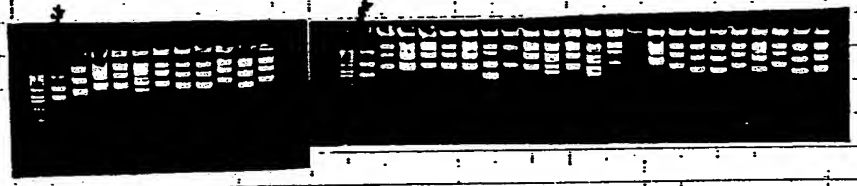
~~2/28/94~~ Monday Pg (66) 58

3/1/94 ~~PM~~ Tuesday

Inoculate into 3ml TB+Amp for Boiling mini preps
Incubate 37°C w/aeration

3/2/94 Wednesday

Do boiling mini preps
Run 5ul on 1% gel with Kb ladder and pBSK



3/3/94 Thursday

PCR Plasmid DNA

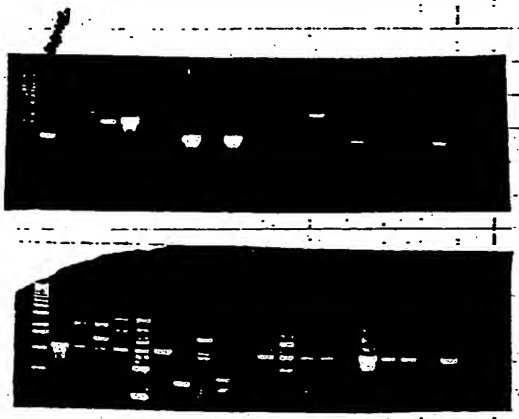
Dilute	1 → 2ml KO	use ul in Rxn
MBF	0.1	
H-TPAN 157-S	0.1	① Control for TPAN 0 & plasmid
10x dNTP	2.1	
10x PCR	2.1	
Taq	0.15	
H_2O	15.45	
DNA	1	
	2ml	

P.U 67

HTLV-I N Screening (Pg 60) Thursday 3/3/94

PCR Program #69.

Run on



- have Sequenced Reverse
- Make internal primers

2, 3, 4, 7, 9, 13, 14, 15, 22, 23, 24, 25, 28, 33

2	S01	13	S06	24	S11
3	S02	14	S07	25	S12
4	S03	15	S08	28	S13
7	S04	22	S09	33	S14
9	S05	23	S10		

HTPAN 03, 04, 13 & 14 look good.

Sequence, with gene specific Primers. 3'→5'

Forward Sequence did not sweep

Re screen

[redacted] 68 HTPAN08 SCREENING

4/19/94 Tuesday

Plate 20 plate
~110,000 plaques/plate

1×10^9 pfu/ml.

0.8 ml phage into 6 ml LE392 OD_{600} =
Incubate 37°C 15 min
Put into Melted LB + Agarose (0.75%)
Plate onto 150 mm NZY Plates
Incubate 37°C 5-6 hrs.
Put at 4°C O/N.

4/20/94 Wednesday

Denature 2 min
Neutralize 5 min

Pd Prehybridize 2 hr - 42°C
at 2x PIPES

Random Prime
HTPAN08 xho/RI

DNA	1 µl	
5x Primers	10 µl	
H_2O	23 µl	
	34	

Heat 100°C 5 min
Quick Chill Spin
Chill on ice

Add	10 µl	5x Buffer dATP
	5 µl	^{32}P dATP
	1 µl	Klenow

HTPAN08 Screening

Wednesday 4/20/94

Incubate 37°C 10 min

Put through G-25 Columns

Count Incl.

~1.3X10^6 50 µl.

Boil 5 min
Chill on ice
Add to 2XPIPES Hyb.
Incubate 42°C O/N

Thursday 4/21/94

Wash 0.2XSSC, 0.1% SDS.

Wash 3X at 65°C
0.2XSSC, 0.1% SDS.

Put on film -80°C
Signal not very strong - So
leave until Monday

Monday 4/25/94

Develop films

HTP- 24
HTN 13

70 + TRANS DB

4/25/94 Monday

Pick plaque onto 300 µl of Sm.

Incubate at Room 4°C ON

4/26/94 Tuesday

PCR Rack Phage

M13F	0.2
157-S	0.2
10x dN	2.5
10x PCR	2.5
H_2O	19.4
Taq	0.7
Phage	5
	25

Program # 58

Do 2° Screens of ⊕ clones

pg 22

HTPAN 08

(pg 70)

Wednesday 4/27/94

Use small NZY plates —
& NO plates so had to make own
NZY Plates *

Thursday 4/28/94

LE392 OD_{600} = 0.5 — 100µl
+ 1:500 Dilution of phage.

Incubate 37°C 6 hrs
Cool at 4°C ON

FRIDAY 4/29/94

Do plate lifts
place orientation marks.

Monday 4/5/94

Denature 2 min 0.5N NaOH / 1.5M NaCl
Neutralize 5 min 0.5M Tris pH 8.0 / 1.5M NaCl
Wet in 2x SSC 5 min
Allow filters to dry

(133)

130 HTPAN08

5/3/94 Tuesday

5

Bake Blots
60°C 1 hour

Make Probe
HTPAN08 Xho/RI

DNA	1 ul
5X Primers	10 ul
H2O	23 ul
	34 ul

Heat 100°C 5 min
Quick Spin
Place on ice
Add

5X dATP Buffer	10 ul
^{32}P dATP	5 ul
Isleno	1 ul
	50 ul

Incubate 37°C 10 min
Put through G-25 column
Count 1 ul

1.0 x 10^6 count/ml ≈ 50 ul

~~Use 200~~
Use 30 ml Hyb Buffer
Prehybridize Blots 1 hr in
3c & PIPES Buffer at 42°C
Pour off Prehyb add the Hyb buffer

Tuesday 5/3/94

Heat
Probe + 50 µl Salmon Sperm DNA
at 100°C 5min
Quick Chill
Add 70 µl to Hyb buffer w/ lifts
Incubate 42°C O/N with
Slight Agitation.

Wednesday 5/4/94

Wash Blots -
Rinse 1X 0.2XSSC/0.1% SDS
Wash 3X in
0.2XSSC + 0.1% SDS
at 65°C

Put No Fdm Cassette
so store at RT in 0.2XSSC & 0.1% SDS

Thursday 5/5/94

Put on film
-80°C for 4 hrs
Develop film
Picked ① clones

5/6/94 ~~Thursday~~ FRIDAY

Have full length

found that 504, 513, 514
fit onto a Contig &
When Blasted against HGS/TIGR
Database returned a Clone
which when blasted against
HGS/TIGR EST Produced a match
to HTPAN.

Sequence to get full length

5/8/94 Make Oligos to sequence
More 3' - forward Forward End

5/10/94 Monday

Submit for Sequencing

5/11/94 Tuesday

Transform DH5α 100 plasmid
& Do maxi Preps.

5/12-5/13 off - Carrie took plates out of 37°C to 4°C (pg 130)

136

HTPAN08

(pJ402 132)

5/16/94 Monday

Pick 8 clones of each
504, 513 & 514 into LB Amp
in 96 well dish.

Incubate 37°C O/N

5/17/94 Tuesday

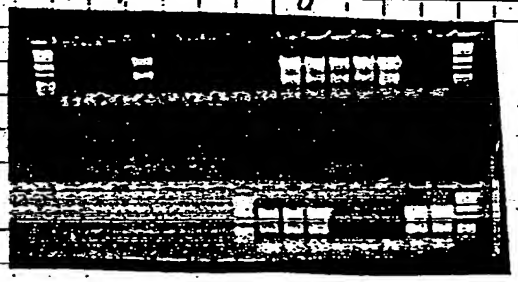
Run PCR. using M13 F & Rev.

		25X
M13F=M13R	1ug/ml	0.1	25
10x dNTP		2	50
10x PCR		2	50
H_2O		13.7	342.5
Taq		0.2	5.0
culture		2	—

Run PCR. Program #69.

95°C 5min
95°C 20sec
55°C 20sec } 30x
72°C 1min
72°C 7.5min.
4°C

Run 1 Dot on gel with 1 Kb ladder



Tuesday 5/17/94

Inoculate 400 ml
TB + Amp with
504 - 3
513 - 5

Incubate 37°C w/ aeration

Wednesday 5/18/94

Maxi Prep

Spin Culture 5K 10 min
Pour off
Resuspend in 15 ml Resuspension Buffer
Add 15 ml Cell Lysis Buffer
Add 15 ml Neutralization Buffer
Spin 8K 10 min
Transfer to Spin Tube
Add Isopropanol to 0.6 Volumes
Mix
Spin 9K 30 min
Pour off
Resuspend pellet in 1 ml TE
Transfer to 2 - 1.5 ml eppendorfs
- 2X Phenol / Sevag
2X Sevag
Ethanol ppt 1/10 Vol 3M Na Act.
2 Vol Ethanol
Ice 15 min
Spin 15 min
Pour off Supernatant
2X 70% Ethanol wash
1X 100% Ethanol Wash
Dry pellet & Resuspend

Read OD 260/280
Run 2 ul on gel with Marker

138 HTPAN08

5/18/94 Wednesday

5/19/94 Thursday

Read $OD_{260}/280$

1:100 Dilution

	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
	0.0011	0.0007	0.0005	4.0362	0.2478	
HTPAN08	0.0679	0.0344	-0.0036	1.8790	0.5322	0.34ug/ul.
HTPAN08S04	0.1344	0.0674	-0.0049	1.9259	0.5192	0.64ug/ul.
HTPAN08S13	0.6327	0.3200	-0.0027	1.9689	0.5079	3.16ug/ul

Submitt for Sequencing

	HTPAN08	HTPAN08S04	HTPAN08S13
	Fc	Fc	Fc
	Rc	Rc	Rc
L157-S	RP01b	RP01	RP01
R318S	RP03b	RP03b	RP03b
R166S	RP05b	RP05	RP05
R338-S	RP06b	RP06	RP06
F488A	RP07b	RP07	RP07
	HTPAN08seq1 pol	RP10	HTPAN08S13R pol RP09
			RP10

Pg 53 book #168
AMC #6

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm		
19mer 1 HE35629	0.0558	0.0297	-0.0001	1.8768	0.5328	0.46ug/ul	73.4 pmol/ul
19mer 2 HPRAC80	0.0508	0.0336	-0.0005	1.5008	0.6663	0.42ug/ul.	67 pmol/ul.

2.2.94

2/7/94

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm		
1 HTPAN08F 3185	0.0473	22mer 0.0364	5124 -0.0044	1.2674	0.7890	0.39ug/ul	59.1pmol/ul
2 HTPAN08R 4050	0.0350	19mer 0.0176 9125	-0.0054	1.7582	0.5687	0.29ug/ul	46.1pmol/ul
3 HTPAN08R 6665	0.0499	19mer 0.0270 5126	0.0009	1.8805	0.5318	0.41ug/ul	65.7pmol/ul

2/14/94

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm		
22mer 1 HKMA61-2055	0.0430	0.0324	0.0065	1.4044	0.7121	0.22ug/ul	37pmol/ul
19mer 2 HUMTN17 -2595	0.0384	0.0237	-0.0000	1.6219	0.6166	0.190g/ul	30pmol/ul

2/15/94

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm
1 5512	0.0300	0.0194	0.0023	1.6231	0.6161
2 5513	0.0324	0.0164	0.0011	2.0444	0.4892

3/4/94

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm		
1 HHBE664 95c	0.0551	22mer 0.0346	0.0015	1.6215	0.6167	0.36ug/ul	50pmol/ul
2 HHRSK38 59c	0.0566	19mer 0.0286	0.0021	2.0539	0.4889	0.37ug/ul	51.6pmol/ul

150

3/8/94

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
11553	0.0691	0.0450	-0.0006	1.5287	0.6541	
25677 HALSK POL	0.0935	21mer 0.0524	0.0017	1.8117	0.5520	89 pmol/ul
35676 HTPAUCB	0.1129	22mer 0.0588	0.0023	1.9576	0.5108	102 pmol/ul
45675 HEBAM POL	0.0750	18mer 0.0526	0.0022	1.4427	0.6931	79 pmol/ul
55670 HCMM POL	0.0827	19mer 0.0505	0.0027	1.6746	0.5972	89 pmol/ul

3/10/94

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
5697 1HE9MF73 19Z	0.0857	21mer 0.0477	0.0010	1.8121	0.5519	81 pmol/ul
5689 2HE35E29 25I	0.0604	20mer 0.0385	-0.0009	1.5565	0.6425	60 pmol/ul
5688 3 HTPAUCB 48B	0.0536	21mer 0.0283	-0.0017	1.8443	0.5422	51 pmol/ul
5685 4 HPL8T52 222	0.0551	20mer 0.0375	-0.0010	1.4573	0.6862	55 pmol/ul
5686 5 HPRAL56 55I	0.0268	18mer 0.0179	-0.0000	1.4972	0.6679	30 pmol/ul

3/16/94

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
5766 1HTPAUCB 12MR	0.0467	19mer 0.0270	0.0018	1.7815	0.5613	61 pmol/ul

3/17/94

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
5780 1TMD186	0.0746	0.0417	-0.0003	1.7828	0.5609	19mer 78pmol/ul
5781 2TMD187	0.0770	0.0555	-0.0005	1.3835	0.7228	19mer 81pmol/ul
5782 3TMD130	0.0762	0.0506	-0.0021	1.4809	0.6752	19mer 80 pmol
5783 4TMD131	0.0847	0.0564	-0.0024	1.4806	0.6754	21mer 80pmol/ul
~~5~~	~~-0.7000~~	~~0.7000~~	~~-0.7000~~	########	########	
5784 6TMD135	0.0465	0.0310	-0.0028	1.4579	0.6859	20mer 46pmol/ul
5785 7TMD137	0.0732	0.0437	-0.0028	1.6350	0.6116	19mer 77pmol/ul
5786 8TMD139	0.0658	0.0527	-0.0019	1.2388	0.8073	19mer 69pmol/ul

3/18/94

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
~~5798~~ 1	~~-0.0007~~	~~-0.0016~~	~~0.0005~~	~~0.6323~~	~~1.8786~~	
5798 2TMD140RPOI	0.0538	18mer 0.0362	-0.0022	1.4574	0.6862	59pmol/ul
5799 3TMD144RPOI	0.0137	18mer 0.0056	-0.0021	2.0592	0.4856	15.2
5800 4TMD147RPOI	0.0111	20mer 0.0045	-0.0031	1.8718	0.5343	11.1 pmol/ul
5809 5HETA576R POI	0.0061	19mer 0.0020	-0.0034	1.7501	0.5714	64 pmol/ul
5810 6HETA594R POI	0.0377	19mer 0.0129	-0.0036	2.5057	0.3991	39 pmol/ul
5811 7HAFB05I R POI	0.0244	20mer 0.0129	-0.0043	1.6674	0.5998	24.4 pmol/ul